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Re: New Bedford Harbor Superfund Site

Dear Counsel:

Enclosed please find comments filed by the undersigned companies on the Final Draft Detailed Analysis of Remedial Technologies for the New Bedford Harbor Feasibility Study. Please include these comments in the files EPA is maintaining as the "Administrative Record" in this matter.

After you have had an opportunity to review these comments, we request the opportunity to meet with you to discuss these further. We will be happy to discuss with you whether you would like any of our technical experts to be present for this meeting.

Very truly yours,

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COMMENTS ON THE FINAL DRAFT
DETAILED ANALYSIS OF REMEDIAL
TECHNOLOGIES FOR THE
NEW BEDFORD HARBOR FEASIBILITY STUDY

Submitted by

Aerovox Incorporated
AVX Corporation
Belleville Industries, Inc.
Cornell-Dubilier Electronics, Inc.
Federal Pacific Electric Co.

June 30, 1988

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EXECUTIVE SUMMARY

Aerovox Incorporated, AVX Corporation, Belleville Industries, Inc., Cornell-Dubilier Electronics Inc., and Federal Pacific Electric Co. (hereinafter "defense group") submit these comments on the Final Draft Detailed Analysis of Remedial Technologies for the New Bedford Harbor Feasibility Study (hereinafter "DART") prepared for EPA.

The findings and conclusions of the DART are important inputs to the RI/FS because this report defines the "technological building blocks" that are to be used in the construction of remedial alternatives for evaluation.

The defense group has two principal concerns with respect to the DART:

- First, the DART rejects one technology that has been shown to have merit in the design of cost-effective remedial alternatives, in-situ biodegradation, and
- Second, the technologies selected in the DART for further analysis--although capable of being assembled into alternatives that span the range of remedial actions specified in appropriate EPA guidance documents--appear to emphasize remedial actions so costly as to be out of proportion to any benefits of site remediation in view of less costly alternatives alone or in combination which may produce equivalent benefits.

These points are, obviously, two sides of the same coin since both errors lead to an insufficient set of alternatives selected for further study.

The DART's failure to retain in-situ biodegradation for further study is a particularly substantial error. This error is based, inter alia, upon the following flawed premises in EPA's analysis:

- in-situ biodegradation can be effective only if means can be found to accelerate the natural rate of biodegradation,
- in-situ biodegradation would be very costly to implement--particularly in terms of monitoring costs,
- in-situ biodegradation has not been successfully applied to river or harbor sediments,
- in-situ PCB biodegradation has not yet been demonstrated in any environment, and
- laboratory studies have failed to demonstrate unequivocally microbially mediated anaerobic dechlorination of PCBs.

These detailed comments contradict these erroneous premises and point out that:

- in-situ biodegradation could be of use in a number of ways apart from that discussed in the DART. In particular, naturally occurring in-situ biodegradation could reduce whatever risks might be present in the "no action" alternative or could be an effective complement to nonremoval technologies,
- in-situ biodegradation would not necessarily be costly to implement and the DART monitoring cost estimates for this approach are overstated,
- in-situ biodegradation has occurred in numerous river/harbor settings, including the Hudson River, NY, Waukegan Harbor, Illinois, Silver Lake, Massachusetts, and the Escambia River, Florida. More importantly, there is evidence that it occurs in New Bedford Harbor, and

- PCB biodegradation has been demonstrated in both field and laboratory studies, as the recent results reported herein show.

These comments also demonstrate that much more information is available about PCB biodegradation than is apparently known to the authors of the DART. Relevant questions/issues remain to be studied, as is common to the other alternatives retained in the DART for further study. But in-situ biodegradation is an attractive candidate, and should be retained for further study.

I. Introduction

EPA has released a final draft report titled "Detailed Analysis of Remedial Technologies for the New Bedford Harbor Feasibility Study" (hereinafter "DART").¹ Preparation of this report is the penultimate step in phase 1 of the New Bedford Harbor (NBH) feasibility study (FS). It is to be followed by a "scoping of remedial alternatives" which will conclude phase 1 of the FS.

The DART was furnished to counsel for Aerovox Incorporated, AVX Corporation, Belleville Industries, Inc., Cornell-Dubilier Electronics, Inc., and Federal Pacific Electric Co. (hereinafter "defense group"). These comments on the DART were prepared after careful review by counsel and their technical experts. The comments in this document are quite detailed and intended to be constructive. As noted in the above summary, the defense group is principally concerned that EPA is making a significant error in rejecting the in-situ biodegradation alternative as a candidate for further study. Reasons for this concern are detailed in these comments. Collectively these reasons show that EPA should not arbitrarily reject in-situ biodegradation from close study and consideration.

¹E. C. Jordan Co., Final Draft Detailed Analysis of Remedial Technologies for the New Bedford Harbor Feasibility Study, EPA Work Assignment Number 04-1L43, EPA Contract Number 68-01-7250, Ebasco Services, Incorporated, November 1987.

II. A. Background and Critique

In broad terms, the purpose of the DART is to identify and preliminarily evaluate remedial "technologies" that will be used in a more detailed scoping of remedial "alternatives" for New Bedford Harbor. In other words, the DART considers technology modules or building blocks that are to be fashioned into remedial alternatives. Thus, for example, some of the so-called removal technologies (various dredging and excavation options) are not necessarily "stand alone," as some treatment and/or disposal of the contaminated sediments might also be employed. (This should be borne in mind when examining the costs given in the DART; the costs given are for each of the various building blocks alone and not for the complete remedial alternative.)

A number of candidate remedial technologies were screened in the DART. Some, such as supercritical water oxidation and in-situ biodegradation, were dropped from consideration. Others, such as hydraulic controls, dredging, and incineration were retained as options for further study in the RI/FS.

The technologies retained for further study and possible inclusion in the design of remedial alternatives are shown in Figure 1. (Figures and tables are found at the end of the text.) The criteria considered in the decision to retain an alternative for further study include:

- (i) effectiveness (reliability, public health, environment),

- (ii) implementation (feasibility, level of development, support requirements, availability, installation, time, safety, monitoring, permitting, legal constraints, impacts on historical and cultural resources),
- (iii) cost (direct/indirect capital costs and operation and maintenance).

Guidance for the screening process can be found in applicable EPA documents.²

EPA's guidance on feasibility studies under CERCLA identifies several types of alternatives that should be considered as part of an RI/FS:³

- (i) alternatives for treatment and disposal at an off site facility,
- (ii) alternatives which attain applicable and relevant federal public health or environmental standards,
- (iii) as appropriate, alternatives which exceed applicable and relevant standards,
- (iv) alternatives which do not attain applicable and relevant health or environmental standards but will reduce the likelihood of present or future threats from the hazardous substances, and
- (v) a "no action" alternative.

²See, e.g., U. S. EPA, Guidance on Feasibility Studies Under CERCLA, EPA/540/G-85/003, June 1985, hereinafter "Guidance 1985." See also, EPA, Guidance for Conducting Remedial Investigations and Feasibility Studies Under CERCLA, Office of Emergency and Remedial Response, Office of Solid Waste and Emergency Response, OSWER Directive 9335.3-01, Draft, March 1988, hereinafter, "Guidance 1988."

³Guidance 1985, pp. 2-16, 2-17.

This guidance has been expanded in a subsequent draft⁴ which supports the consideration of such a broad range of alternatives, introduces some new material on ARARs, and reflects SARA emphasis on alternatives that "permanently and significantly reduce" the volume, toxicity, or mobility of hazardous substances, pollutants, and contaminants."

Although the range of alternatives that can be developed from the technologies shown in Figure 1 is arguably consistent with the above guidance, the relative emphasis on remedial technologies selected for further evaluation appears to be skewed towards the alternatives that are most expensive. For some combinations of remedial technologies total remedial costs could approach \$1 billion, a figure wholly disproportionate to any possible benefits from site remediation. Other effective but lower-cost alternatives (represented broadly by the nonremoval technologies) identified in the DART are relatively few in number, and not extensively discussed. As a rough indicator of relative emphasis, a total of 61 pages in the DART is devoted to nonremoval alternatives as compared with more than 416 pages to the remainder. These statistics are at least suggestive of the relative emphasis placed by EPA on remedial technologies that happen to be the most expensive.

⁴Guidance 1988

Thus, the defense group is concerned that there will be a lack of objectivity in the RI/FS for this site and that the alternatives are already being structured so as to call for an inefficient and very expensive remedial option. Similar concerns were detailed in earlier defense group comments on the proposed New Bedford Harbor Pilot Dredging Program.

1. Tentative Nature of DART Findings and Implications for Critique

It should be noted that the analysis contained in the DART is acknowledged to be preliminary in a number of key respects. For example:

- (i) as noted, technology modules are not complete remedial alternatives,
- (ii) there is no explicit consideration of whether or not the ARARs can be attained (DART at p. 3-1) by the use of a particular technology, rather this topic is to be addressed in the detailed analysis of remedial alternatives (phase III of FS process),
- (iii) explicit calculation of benefits is likewise deferred (see, e.g., DART at p. 4-35) until the remedial alternatives are identified,
- (iv) finally, cost estimates are only preliminary (see, e.g., DART at p. 4-42, p. 4-43) and subject to revision. Some cost estimates have not been developed (see, e.g., DART at p. 8-38).

Further study later in the RI/FS process offers the opportunity to sharpen judgments, reduce uncertainties, and correct

any errors with respect to the analysis of the alternatives retained by EPA for further study. Errors or misjudgments with respect to technologies retained for further study are potentially "reversible" or self-correcting.

But other possible decisions in the DART are not reversible, particularly with respect to alternatives that were either not considered or were considered but rejected in the DART. Rather than address all the possible flaws in judgments or estimates in the DART that will have the opportunity to be revised on further study, the defense group believes that it is more constructive to focus at this time on conclusions reached in the DART that, unless given additional consideration, will not be subject to further review. Thus, the thrust of these comments is on irreversible errors in the DART--particularly the omission of in-situ biodegradation as a potentially cost-effective alternative that merits further study.

2. DART Perspectives on In-Situ Biodegradation

The DART report (DART at E-6, also pp. 4-46 to 4-49) categorically rejects in-situ biodegradation as a candidate remedial technology. It proposes a very narrow and incomplete definition of this approach (DART at pp. 4-46, et seq.) as

"In-situ biodegradation is accomplished by enhancing the biodegradation capabilities of either the indigenous microbes and/or exogenous sources of microbes,"

a concept repeated and emphasized in a 29 Feb. 1988 letter to defense counsel.⁵ As noted below, the defense group maintains that a much broader concept of in-situ biodegradation is appropriate.

With respect to the effectiveness of in-situ biodegradation, the DART erroneously notes (p. 4-47) that "in-situ biodegradation has never been successfully applied to river or harbor sediments."⁶ It also (p. 4-47) erroneously states that "in-situ PCB biodegradation has not yet been demonstrated in any environment," an assertion refuted by laboratory and field data presented in these comments.

As to implementability, the DART claims that (p. 4-47), "there is much conflicting evidence regarding the occurrence and mechanisms of in-situ biodegradation of PCBs; therefore, the full range of factors involved with implementing this technology is unknown," and suggests that the required research would be too difficult or time-consuming to be accomplished within the scope and time constraints of this project.

⁵From Roger J. Marzulla, Assistant Attorney General, Land and Natural Resources Division, by Ellen M. Mahan, Attorney, Environmental Enforcement Section.

⁶Such a statement presumably is based upon the narrow "enhancement" construction of the in-situ biodegradation definition. As discussed at length in these comments--and, indeed, in the DART itself--there are numerous instances of river/harbor systems where biodegradation of PCBs has been reported to occur.

Finally, although no cost estimates are presented, the DART speculates that construction and implementation costs associated with this approach would (DART at p. 4-48), "probably be comparable to other technologies evaluated...whereas the costs associated with monitoring, sampling, and analysis would likely far exceed those for any other technologies."

Based upon the foregoing, the DART concludes that (p. 4-48), "in-situ biodegradation should be eliminated from further consideration as a treatment technology for New Bedford Harbor sediments."

3. DART Perspectives on Biodegradation As Treatment Technology

Although in-situ biodegradation is rejected in the DART, "advanced biological methods" are retained for further evaluation as a treatment technology. The reasons for rejection of in-situ biodegradation while retaining "advanced biological methods" are not made explicit. But the apparent rationale relates to the "controllability" of a specially constructed treatment system and perhaps to the speculation (DART at p. 6-4) that aerobic (in the presence of oxygen) degradation systems would be more effective than anaerobic (without oxygen) systems--a conjecture that the defense group disputes. Significantly, many of the literature citations in Chapter 6 of the DART (pp. 6-1 et seq.) refer to biodegradation in river/harbor sediments--which references are

directly at odds with the conclusions expressed earlier in Chapter 4 of the DART (p. 4-47).

B. Comments of the Defense Group on In-Situ Biodegradation

This section summarizes the comments of the defense group with respect to the examination of in-situ biodegradation in the DART.

1. DART Definition of In-Situ Biodegradation Excessively Narrow

First, the defense group rejects the definition of in-situ biodegradation employed in the DART as excessively narrow. An improved characterization of the possible role/utility of in-situ biodegradation at NBH should consider at least three elements.

- First, evidence at NBH and other river/harbor systems and in the laboratory (see below) indicates that biodegradation of PCBs can occur in sediments even absent intervention and control to "enhance the biodegradation capacity." This phenomenon is important in the evaluation of a "no action" alternative because, over time, naturally occurring biodegradation will remove the potentially more biologically active congeners, and hence, lower whatever perceived health risks are alleged to be attributable to PCBs that are present at New

Bedford Harbor in the absence of any remedial action.

- Second, in-situ biodegradation may be an important element in the evaluation of other remedial options, particularly the so-called nonremoval options identified in Figure 1. A purported liability of most of these technologies noted in the DART (e.g., at p. 4-45 for the case of hydraulic controls) is that these options are inconsistent with the preference in the SARA guidelines for clean-up alternatives that "permanently and significantly reduce mobility, toxicity, or volume of a given waste." Although the permanence of the reduction through capping or hydraulic controls alone may be a legitimate subject of inquiry, in-situ biodegradation may well provide an intermediate and long-term reduction in the toxicity of the isolated sediments. Thus, the combined alternative of nonremoval technologies and naturally occurring biodegradation could be fully consistent with SARA preferences. This aspect of nonremoval technologies has not been considered in the DART, a significant omission.

- Third, there may indeed be possibilities for enhancement of the rate of in-situ biodegradation.

The DART document addresses only the last of the possibilities offered by in-situ biodegradation, and overlooks the first two identified above.

2. DART Criteria for Technology Selection Unevenly Applied

As noted above, the DART rejects in-situ biodegradation as a viable technology, allegedly because inter alia, (DART at p. 4-47), "in-situ biodegradation has never been successfully applied to river or harbor sediments" and later claims that "in-situ PCB biodegradation has not yet been demonstrated in any environment." Not only are these statements incorrect, but also the DART is inconsistent in its application of these same criteria to other technologies. For example, the DART concedes (p. 6-93) that "KPEG is not a proven process" and acknowledges (p. 6-87) that "there has been only limited success of KPEG on soils and sediments." Nonetheless, the DART retains this technology for consideration. Similarly, the DART retains vitrification for further study even though (DART at pp. 6-80 et seq.) it acknowledges that this "technology has not been demonstrated for sediments, and significant questions remain in determining operating costs."

Logical consistency would require that either all of these "undemonstrated" technologies be rejected or all accepted for further consideration.

3. DART Evaluation of In-Situ Biodegradation Cursory and Flawed

The basic approach taken by the authors of the DART toward how biodegradation must be used at New Bedford Harbor is one of complete control of a reactor process--what might be termed the "engineering" philosophy. This overlooks the many successful examples of use of microbial processes that do not require such extensive supplementation, control, or monitoring of the natural process. In nature, for example, the Rhizobium inoculation of legumes and mycorrhizal inoculation of pine seedlings, or in contained environments the production of yogurt, or sourdough bread are accomplished without "precise" controls. Thus, monitoring of populations, nutrients, and most physical chemical parameters (DART at p. 4-47) is not necessary and would not be expected to be necessary in this particular PCB case. For example, in the successful study of PCB degradation in Hudson River sediments, discussed below, nothing other than decreases in PCB congener concentrations needed to be monitored. Thus, the monitoring costs associated with in-situ biodegradation are overstated in the DART.

The engineering philosophy of complete control is probably derived from the presumption that virtually all molecules must be verified as treated and that the rate of biodegradation must be driven to its maximum. Imposition of these requirements comes at a high cost relative to the additional benefits gained. This approach may also be inconsistent in this particular situation with Superfund mandates.

As noted above, the DART separates in-situ biodegradation from contained biological treatment--a distinction which is artificial and premature at this stage. For example, if reductive dechlorination can be made to work for PCBs in sediments, then the option of using in-situ biodegradation in some form versus use of biodegradation technologies when sediments are pumped into a constructed container, ought to be evaluated.

Implementation of biological treatment techniques represents a continuum from a highly contained, monitored reactor (as is more commonly used for high-value products) to a minimal in-situ manipulation. If the microbiological capacity is demonstrated for effective degradation, where it is implemented may be less important and may be a decision more appropriately based on comparative costs.

The DART also seems to be inconsistent in that biodegradation treatment technology and needed work are proposed (DART at p. 6-5) but similar needed work when described under in-situ remedia-

tion (DART at p. 4-48) is considered to be too extensive. The needed microbial research is similar regardless of the place of implementation.

The DART contends that the GE PCB-degrading microbes are incapable of growth in a marine environment (DART at p. 4-47). This contention is pure speculation; in fact, GE researchers have not conducted this experiment in the laboratory (Ron Unterman, GE, personal communication). Indeed, GE's field studies (discussed below) indicate that biodegradation has, in fact, occurred in marine environments; at New Bedford Harbor (Brown and Wagner, 1987) and elsewhere. Furthermore, in section 6.1 of the DART (p. 6-4) it is assumed that contained treatment would require desalination for the active microorganisms to survive. This assumption is wrong on two counts: first, terrestrial microorganisms often grow in the modest salinity of ocean water,⁷ and second, other marine microorganisms may exist that are capable of destroying PCBs. The existence of ecologically equivalent microorganisms in marine and freshwater sediments is well recognized.

⁷This is understandable since wetting and drying of soils often exposes the organisms to a more salty environment than the ocean. Even if PCB-degrading terrestrial organisms were found to be sensitive to ocean water, it ought to be possible to isolate salt-tolerant PCB-degrading variants since resistances are the easiest genetic change to successfully select for in bacteria.

The costs of in-situ remediation are not reasonably presented in the DART; they simply are assumed to be as expensive as other technologies (DART at p. 4-48). The absence of a cost analysis for in-situ biodegradation in the DART is puzzling; other technologies, including even those that were rejected (e.g., supercritical water oxidation) were subject to cost analysis. In comparison, the evaluation of the costs of in-situ biodegradation in the DART is cursory. Although the sampling, monitoring, and analysis costs as they are presented in the DART could be greater for in-situ treatment than for other technologies, it is doubtful that these costs would approach the much greater costs of physical transport of large volumes of sediment and its proper storage, as is required for some of the technologies retained for further evaluation.

The discussion in the DART on Advanced Biological Methods (pp. 6-1 to 6-6) is uneven, full of inconsistencies, and reveals a basic misunderstanding of research findings relative to PCB biodegradation. For example, the DART equates (p. 6-2) 2,3-dioxygenase activity with the anaerobic dechlorinating system. These are two distinctly different systems. In confusing these two systems, the DART gives the misleading impression that the two activities are redundant. They are not. The 2,3-dioxygenase requires oxygen and catalyzes one possible initial step in the aerobic degradation of PCBs with adjacent unsubstituted ortho and

meta carbons; its activity is further restricted to PCBs with generally fewer than six chlorines. In contrast the dechlorinating system is anaerobic--functioning in the absence of molecular oxygen. The Hudson River dechlorinating microorganisms are most active in removing meta and para chlorines, but the environmental data for Silver Lake (Massachusetts) suggest there are also organisms capable of removing ortho chlorines (Brown et al., 1987a, b). The dechlorination of the more highly chlorinated PCBs (i.e., more than six chlorines) results in congeners that are more aerobically biodegradable by 2,3- and 3,4-dioxygenase enzyme systems.

As a second example, the DART states (p. 6-3) that "lab studies have failed to unequivocally demonstrate microbially mediated anaerobic dechlorination of PCBs." In a following section detailed data are presented which unequivocally demonstrate that microbially mediated dechlorination of PCBs does occur.

As a third example, the DART states (p. 6-6) that "[I]t is unlikely that complete degradation of PCBs could be attained because...the di-ortho chlorinated PCBs and PCB congeners with six or more chlorines are resistant to degradation." In making this statement, the DART authors are ignoring the existence of 3,4-dioxygenase (although they mention it on p. 6-2) and the fact that dechlorination yields more readily aerobically degradable

PCB congeners (although they mention this on p. 6-5). Current evidence shows no Aroclor congener to be totally resistant to both anaerobic and aerobic attack. Some may have slower degradation rates than others, but as stated by Bedard et al. (1987), "the constituents of every congener peak in Aroclor 1242 were transformed by dechlorination, oxidation, or a combination of both...Aroclor 1242 could be completely degraded by a combination of both transformations."

Given the current state of knowledge of both aerobic and anaerobic PCB metabolism, the time scale for bench testing (DART at p. 6-14) of four to six months may be unrealistic. More importantly, the proposed research in the DART may not be worth the effort because the information gained will be little different than what is known now, i.e., partial elimination of the lesser chlorinated PCBs by aerobic bacteria can occur. What is needed is to understand how to improve PCB bioavailability to PCB degrading organisms and to develop the anaerobic dechlorination system--perhaps to attempt to couple it with aerobic degradation so that complete degradation of most of the PCBs will occur. This could take longer than six months; the delay must be evaluated against the potential usefulness of what appears to be a new breakthrough in PCB-destruction technology.

4. Dart Fails to Consider Important Research Results

Finally, the DART demonstrates an insufficient appreciation of research results in reaching the conclusion that little is known of PCB biodegradation. The technical material summarized below shows that, contrary to the assertions in the DART, a great deal is known about biodegradation of chloroaromatic compounds generally, and PCBs in particular. Moreover, evidence that PCB degradation takes place in sediments--purportedly lacking according to the DART--is now available as is discussed in Section III.

III. Background on Anaerobic Biodegradation

Considerable work has been done with respect to the biodegradation by reductive dechlorination of a wide variety of chloro-aromatic and chloroaliphatic compounds. Because chlorine substitution often blocks degradation by conventional pathways, the value of this process is that it removes chlorine as the initial step, often making the compound more susceptible to further degradation by other biological or chemical processes. This section of the DART comments summarizes information on aromatic reductive dechlorination especially relevant to PCBs and the New Bedford Harbor site.

A. Range of Pollutant Chemicals Degraded

Several investigators have been conducting research in the area of anaerobic biodegradation and dehalogenation. For example, Tiedje and others (Horowitz et al., 1982 and Shelton and Tiedje, 1984a) have screened more than 100 different chemicals for biodegradation in anaerobic sludge or eutrophic lake sediments. The classes that were biodegraded include chlorinated aromatic compounds (Suflita et al., 1983), cresols (Shelton and Tiedje, 1984a; Boyd et al., 1983), phthalates (Shelton et al., 1984), chlorinated hydroxylated methoxylated benzenes (Woods 1985), and polyethylene glycols (Dwyer and Tiedje, 1983). The anaerobic degradation of chlorinated chemicals is of particular

interest because of the widespread presence of these chemicals (e.g., PCBs, TCDD (2,3,7,8-tetrachlorodibenzodioxin), chlorobenzenes, PCP (pentachlorophenol)). Furthermore, the key reaction that has been observed--replacement of the aromatic chlorine(s) with hydrogen, hence the term reductive dechlorination--was new and a particularly promising biotransformation. In many cases if a chlorine atom is removed, the compound becomes both more biodegradable and less biologically active. Aerobic metabolism of highly chlorinated aromatic chemicals--the approach recommended in the DART--is often restricted since two adjacent ring positions must be free for hydroxylation; the Cl is removed only after ring hydroxylation and opening. Thus, the anaerobic dechlorination which occurs prior to ring opening provides a means to overcome the block preventing aerobic degradation by providing the necessary free ring carbons--which suggests the possible merits of a sequential anaerobic/aerobic approach. Reductive dehalogenation has been known since 1967 but the previous evidence was limited to the removal of chlorine from nonaromatic carbon atoms (Tiedje et al., 1987).

Since the initial discovery of aromatic dehalogenation (Suflita et al., 1982) evidence of dehalogenation has expanded to include chemicals in the following classes of aromatic compounds: chlorinated benzoates (Suflita et al., 1983; Shelton and Tiedje, 1984b), chlorinated phenols and especially pentachlorophenol

(Mikesell and Boyd, 1985, 1986), chlorinated benzenes (e.g., hexachlorobenzene, Fathepure et al., 1988, Tiedje et al., 1986), chlorinated phenoxy herbicides (e.g., 2,4,5-T) (Suflita et al., 1984; Mikesell and Boyd, 1985), and chlorinated dihydroxybenzenes (Fathepure et al., 1987). In addition there is reductive aryl-dechlorination reported in flooded soils for the pesticides techlofthalam, diuron, benthocarb, and chloronitrofen (summarized in Tiedje et al., 1987). As described in detail below there is now evidence for the anaerobic dechlorination of PCBs under laboratory conditions. Figure 2 summarizes, with examples, the classes of chemicals for which reductive dechlorination has been observed.

In short, it is becoming apparent that most chlorinated chemicals can be reductively dechlorinated by appropriate anaerobic communities, and that complete persistence is rare. Furthermore, the communities capable of reductive dechlorination seem to be widespread. Thus, in-situ reductive dechlorination in sediments is to be expected in New Bedford Harbor.

B. Basic Studies on Chlorobenzoate Dechlorination by DCB-1

A number of species biodegrade halogenated chemicals. One microbe, DCB-1, has been isolated in the laboratory. Information relative to this bacterium is presented in Appendix A. This information is of interest because it suggests why microorganisms

may be selected to dechlorinate aromatic compounds--they may gain energy for growth as well as new electron acceptors--usually the most limiting factors for organisms in anaerobic environments.

Thermodynamic calculations of Tiedje and others for most classes of aromatic compounds including the PCBs show that in every case considerable energy is available from each dechlorination event (Brown et al., 1987b, see also J. Dolfig, K. Harrison, J. Tiedje, unpublished data). Natural selection should work in the direction of selecting organisms or mutant strains that can use this energy for growth. Thus, it would not be surprising if long-term PCB exposure effected selection of PCB dechlorinating organisms.

C. Dechlorination of PCBs and the Closely Related Halobenzenes

1. Dehalogenation of Benzenes

Findings on the dehalogenation of hexachlorobenzene (HCBz) and hexabromobenzene (HBBz) closely parallel recent discoveries concerning the dechlorination of PCBs. These chemicals are analogous to PCBs in being halogenated aromatic compounds without polar functional groups and show that the dehalogenation of highly halogenated (saturated) carbon rings is possible. Also, recent bench scale anaerobic upflow reactors suggest that engineered biological destruction of compounds such as HCBz may

indeed be feasible--a conclusion at odds with the speculation in the DART.

As with PCBs, the first evidence of the anaerobic dehalogenation of HCBz came from environmental samples. As noted by Bailey (1983), the data of Oliver and Nicol (1982) on the distribution of chlorobenzenes in the Great Lakes sediments are suggestive of environmental dechlorination of HCBz. These researchers reported that the ratio of di- and trisubstituted chlorobenzenes to penta- and hexachlorobenzenes increased dramatically with depth in the sediment column. This is consistent with dechlorination in the anaerobic zone.

The biological dechlorination of HCBz in anaerobic sludge and sediment has been confirmed in laboratory experiments (Fathepure et al., 1988) as discussed in Appendix B.

2. Dechlorination of PCBs

a. Evidence from Field Analyses

GE scientists have reported evidence from environmental sampling that dechlorination of PCBs occurs in contaminated sediments. This evidence comes from the analysis of PCB congener profiles in sediment core samples taken from the Hudson River, New York, Silver Lake, Massachusetts (Bopp et al., 1984; Brown et al., 1984, 1987a, b), New Bedford Harbor, Massachusetts (Brown and Wagner, 1987), and Waukegan Harbor, Illinois (Stalling,

1982). There is similar but yet unpublished evidence for Aroclor 1254 dechlorination in the Escambia River, Florida (O'Connor, personal communication to Dr. John Quensen).

PCB congener profiles have been determined for cores taken from the Hudson River between Thompson Island Dam and Ft. Edwards. The major sources of PCBs in this region of the river were the GE capacitor plants at Hudson Falls/Ft. Edwards. Because one PCB mixture (Aroclor 1242) was the most important PCB input to this section of the river for more than 20 years, it is possible to compare to the original mixture the relative proportions of different congeners in sediments of different ages (Brown et al., 1984, 1987a, b). Moreover, even if the sources were mixed it is also possible to distinguish reductive dechlorination by comparing the proportion of ortho chlorines in the residue to meta and para chlorines because ortho chlorines are more resistant to dechlorination. As dechlorination proceeds, the ratio of meta and para to ortho chlorines decreases.

Such comparisons reveal that the congener distribution patterns in the deeper anaerobic sediments are very different from those for the aerobic near-surface sediments (Figure 3). The anaerobic sediments show decreases for the more highly chlorinated congeners and increases for the lesser chlorinated congeners, especially those with ortho substitutions. There are differences between cores as to which congeners are most readily

degraded. This specificity suggests that there may be several types of microorganisms inhabiting the different sediments that are capable of anaerobic dechlorination.

Congener pattern changes with depth (age) in the same core indicate a stepwise dechlorination. The ortho substituted products (e.g., 2-,2,2'-,2,6-,2,2',6-, and 2,2',6,6'-PCBs) tend to accumulate in anaerobic sediments.

Similar conclusions can be drawn from the analysis of PCB congener profiles in cores taken from Waukegan Harbor. In this case, Aroclor 1248 from the manufacture of outboard motors was the major input. Raw data for the five cores was compiled by Stalling (1982) and interpreted by Brown et al. (1987a). Some peak assignments are uncertain because a different (Apolane) capillary column was used, but it is evident that penta-PCBs (especially in two samples) and tetra-PCBs (in three samples) decreased markedly while an enrichment for di- and trisubstituted congeners was noted. In general, the dechlorination capability seemed weaker than for the Hudson River sediments, and some probable differences in congener selectivity were noted. Most significant, however, was the marked decrease in relative amounts of the more biologically active congeners 3,3',4,4'-CB, 2,3,3',4,4'-CB, and 2,3',4,4',5-CB (>98% decrease).

Substantial dechlorination of PCBs was indicated in 11 of 12 cores taken in the upper estuary region of the Acushnet River,

Massachusetts (Brown and Wagner, 1987). The PCBs in this region of the river are reported primarily as Aroclor 1242 and 1254. Total PCB concentrations reportedly ranged from 0.3 to 3,775 ppm on a dry weight basis.

The evidence for dechlorination in these samples is that the ratio of ortho Cl to meta plus para Cl is higher than expected for any combination of Aroclors 1242 and 1254. Indicator peaks for both Aroclor 1242 (2,3,3',4'-CB) and 1254 (2,2',3,4,5'-CB) averaged 1.6 half-losses (approximately a 67% reduction) over a 10- to 20-year period.⁸

b. New Evidence from Laboratory Studies

Not only do the GE sediment congener analyses reveal biological dechlorination of PCBs, but so also do controlled laboratory experiments. Tiedje and Quensen have conducted three different types of laboratory experiments to evaluate PCB dechlorination in sediments; all showed PCB dechlorination. These experiments directly contradict the conclusions of the DART and merit discussion in detail. A summary of these results is presented here. Appendix C contains additional details.

⁸One half-loss is equivalent to a 50% reduction in concentration, two half-losses to a 75% reduction, etc. The number of half-losses, X, corresponding to a reduction of concentration to a fraction C/C_0 of the original concentration is, $X = -1.443 \ln (C/C_0)$.

The first of the Tiedje and Quensen experiments involved the addition of five pure PCB congeners to Hudson River sediments, sewage sludge, and chlorophenol-enriched sewage sludge. The five congeners were selected from among those not present in Aroclor 1242 in any appreciable amount so as to minimize any likelihood of "masking" from any Aroclor 1242 present in the sediments. Chemical analyses of these samples over time enabled the course of biodegradation to be monitored by the decrease in concentration of the added congeners and the increase in concentration of the dechlorination products from these congeners. All samples exhibited some dechlorination, but the Hudson River samples exhibited the highest rates.

The second series of experiments involved the actual transfer of microorganisms from contaminated Hudson River sediments and other river sediments to nonPCB-contaminated sediments and the addition of five pure PCB congeners to these mixtures. Here again dechlorination was evident, demonstrating the viability of the transfer process in the laboratory.

Finally, a third experiment involved a similar transfer, but Aroclor 1242 (at concentrations of 14, 140, and 700 ppm) was added rather than the five indicator congeners. The purposes of this experiment were to demonstrate unequivocally that biodegradation of congeners present in Aroclor 1242 would occur (i.e., to eliminate any argument to the effect that only the five indicator

congeners were capable of being biodegraded) and to study the dependence of the rate of dechlorination on the Aroclor 1242 concentration. This experiment again demonstrated biodegradation. The rate of dechlorination was shown to be concentration dependent in this series of experiments--the highest rate corresponding to the highest PCB concentration.

The PCB dechlorination observed in these laboratory experiments is, in all likelihood, biologically mediated. All dechlorinations observed in the pure PCB congener experiments involved the removal of meta- or para- chlorines only and in the Aroclor 1242 experiment ortho- only substituted congeners accumulated. Both of these observations are consistent with GE's environmental data (Brown et al., 1984, 1987a, b) showing the accumulation of ortho-only substituted chlorobiphenyls in Hudson River sediments. The preferential removal of the meta and para chlorines is particularly significant since these compounds are more biologically active (McKinney and Singh, 1981; Safe et al., 1982), and more resistant to aerobic degradation (Brown et al., 1984). Conversion of these congeners into those that are degradable under aerobic conditions (Bedard et al., 1987) is also to be expected.

IV. Reductive Dechlorination at New Bedford Harbor

A. Evidence That PCB Biodegradation Occurs in New Bedford Harbor

The congener analysis of the sediment profile which shows selective loss of nonortho chlorines, plus the disappearance of higher chlorinated PCBs and the increase of lower chlorinated PCBs in most of the upstream cores provides convincing evidence that PCB dechlorination occurs in New Bedford Harbor.

Even in the absence of direct evidence it is reasonable to postulate that the same situation exists as in the Hudson River. There the dechlorinating organisms have been shown to currently reside in the sediments, and they are very active in PCB dechlorination when placed into a clean sediment with new Aroclor 1242.

B. Implications of These Findings for Remedial Action Alternatives

The laboratory finding of substantial anaerobic dechlorination of Aroclor 1242 within 16 weeks by Hudson River microorganisms establishes microbial reductive dechlorination as a potentially valuable remediation technology since the more highly chlorinated congeners that are resistant to aerobic dechlorination are readily dechlorinated. The finding of numerous sediment profiles which show PCB dechlorination, including those from New

Bedford Harbor, show that this process is widespread. Tiedje and Quensen's recent dramatic findings of Aroclor 1242 dechlorination provide a major impetus to rethink PCB remediation schemes at New Bedford Harbor.

The material presented above shows that the key reasons cited in the DART for rejection of in-situ PCB biodegradation are substantially incorrect. In-situ PCB biodegradation has substantial promise either as a stand alone remedial technology or in concert with other nonremoval alternatives. There is field evidence that in-situ PCB biodegradation occurs in New Bedford Harbor and both field and laboratory evidence that PCB biodegradation occurs in other river sediments. The costs of in-situ PCB biodegradation are overstated in the DART. In sum, although more research on this technology is appropriate--as with many of the other technologies retained in the DART--in-situ biodegradation is an attractive candidate and ought to be retained for further detailed study.

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TABLE 1. DISAPPEARANCE OF ADDED PCB CONGENERS
AND APPEARANCE OF DECHLORINATED PRODUCTS
AFTER INCUBATION OF HUDSON RIVER SEDIMENTS

PCB Congener Added	Average % Reduction of PCB ^a		Average % of Original PCB Identified As Products
	Sterile	Alive	
2,2',3,3',4,4',5,5' ^b	std	std	0
2,2',3,4,4',5'6	0.9	12.4	4.1
2,2',4,4',6,6'	-14.6	7.4	?
2,3,4,5,6	-0.2	43.8	≥28.0
2,3',4,4'	-13.3	31.0	?

^a the difference in mean quantities between 8 and 51 weeks, as a percent of the original amount.

^b because no products from this congener were detected, it was used as an internal standard to quantify the amounts of the other congeners in the samples.

TABLE 2. PERCENTAGE OF 2,3,4,5,6-PENTACHLOROBIPHENYL
DECHLORINATED BY MICROORGANISMS FROM VARIOUS SOURCES

Source	Treatment		Week					
			0	4	8	16	24	32
Pine River	Live	\bar{X}	0.0	2.9	4.9	7.2	10.3	10.2
		$S_{\bar{X}}$	0.0	0.2	0.3	0.3	2.0	0.7
	Dead	\bar{X}	0.0	0.0	0.0	0.0	0.0	0.3
		$S_{\bar{X}}$	0.0	0.0	0.0	0.0	0.0	0.3
Silver Lake	Live	\bar{X}	0.0	0.0	0.0	4.6	11.8	13.1
		$S_{\bar{X}}$	0.0	0.0	0.0	0.2	0.6	1.0
	Dead	\bar{X}	0.0	0.0	0.0	0.0	0.0	0.0
		$S_{\bar{X}}$	0.0	0.0	0.0	0.0	0.0	0.0
Red Cedar River	Live	\bar{X}	0.0	0.0	0.0	0.2	1.5	1.3
		$S_{\bar{X}}$	0.0	0.0	0.0	0.2	0.5	0.3
	Dead	\bar{X}	0.0	0.0	0.0	0.0	0.0	0.0
		$S_{\bar{X}}$	0.0	0.0	0.0	0.0	0.0	0.0
Thompson Island	Live	\bar{X}	0.0	0.0	2.2	7.4	17.7	18.1
		$S_{\bar{X}}$	0.0	0.0	0.3	0.4	2.0	1.2
	Dead	\bar{X}	0.0	0.0	0.0	0.0	0.0	0.0
		$S_{\bar{X}}$	0.0	0.0	0.0	0.0	0.0	0.0
H7	Live	\bar{X}	0.0	0.0	2.2	6.8	13.9	12.7
		$S_{\bar{X}}$	0.0	0.0	0.1	0.0	0.8	0.2
	Dead	\bar{X}	0.0	0.0	0.0	0.0	1.3	2.8
		$S_{\bar{X}}$	0.0	0.0	0.0	0.0	1.3	2.8

Thompson Island and H7 are PCB-contaminated sites in the Hudson River, New York.

TABLE 3. RETENTION TIMES AND STRUCTURES FOR THE
60 PCB PEAKS QUANTITATED IN THE AROCLOR 1242
EXPERIMENT

Peak #	Retention Time (min)	Structure		
1	14.090	2-		
2	17.433	4-		
3	19.378	2-2-	26-	
4	21.677	24-	25-	
5	22.662	2-3-		
6	23.181	23-	2-4-	
7	25.007	26-2-		
8	26.353	3-3-		
9	26.860	3-4-	(34-)	
10	27.227	25-2	4-4-	
11	27.366	24-2-		
12	28.189	26-3-	(236-)	
13	28.934	26-4-	(23-2)	
14	29.896	35-2	(235-)	
15	30.195	26-26		
16	30.765	25-3		
17	30.950	24-3-		
18	31.569	25-4-		
19	32.462	34-2-		
20	32.616	25-26		
21	33.009	24-26		
22	33.153	23-4-		
23	33.604	236-2-		
24	34.347	23-26-		
25	34.977	25-25-	26-35-	
26	35.197	235-2-		
27	35.336	24-25-		
28	35.583	24-24-		
29	36.797	23-25-	(246-26-)	
30	37.044	34-4-	236-3-	(23-24)
31	37.719	25-35-		
32	37.883	236-4-	234-2-	26-34-
33	38.252	24-35	(236-26-)	
34	38.588	23-23-		
35	38.861	235-3-	(246-25-)	
36	39.252	246-24-	(245-3-)	
37	39.757	235-4-	(235-26)	(23-35-)
38	40.090	245-4-		
39	40.489	345-2-	25-34-	(235-24-)

TABLE 3.
(continued)

Peak #	Retention Time (min)	Structure		
40	40.765	35-35-	(245-26-)	(236-25)
41	41.418	236-24-	(246-25-)	(234-3-)
42	42.250	23-34-	234-4-	
43	42.569	235-25-	236-23-	(246-246-)
44	42.698	234-23-		
45	43.073	235-24-	245-25-	
46	43.508	245-24-		
47	44.069	2356-3-	(236-246-)	(246-34-)
48	44.469	235-23-	(345-3-)	(2346-4)
49	44.936	245-23-	(2345-2-)	(2356-26-)
50	45.409	234-25-	2346-4-	235-35-
51	45.746	234-24-		
52	45.921	245-35-	236-236-	
53	46.297	236-34-	(34-34-)	
54	47.222	234-23-		
55	47.448	2356-25-		
56	47.978	235-236-	2346-25-	
57	48.526	2345-3-	236-245-	245-34-
58	48.694	2346-24-	234-246-	
59	49.592	345-23-	235-235-	
60	50.970	234-34-	236-236-	

Parentheses denote possible minor components of a peak.

TABLE 4.

Molar percentages of PCBs of different degrees of chlorination over time. Values are the means of two replicates receiving 700 ppm of Aroclor 1242.

Congeners	Weeks			
	0	4	8	16
Live:				
Mono-	0.0 \pm 0.0	1.7 \pm 0.5	50.1 \pm 10.7	66.7 \pm 3.4
Di-	9.1 \pm 0.7	15.3 \pm 0.2	25.5 \pm 3.7	21.3 \pm 0.6
Tri-	48.5 \pm 0.1	48.2 \pm 1.3	16.2 \pm 4.9	8.5 \pm 2.3
Tetra-	36.3 \pm 0.6	30.0 \pm 0.9	6.8 \pm 1.8	3.0 \pm 0.4
Penta-	5.2 \pm 0.2	4.2 \pm 0.2	1.3 \pm 0.4	0.5 \pm 0.1
Hexa-	0.9 \pm 0.0	0.6 \pm 0.0	0.2 \pm 0.1	0.0 \pm 0.0
Autoclaved:				
Mono-	0.9 \pm 0.3	1.2 \pm 0.2	1.7 \pm 0.2	4.0 \pm 0.4
Di-	11.6 \pm 0.8	12.9 \pm 0.1	13.2 \pm 0.4	15.3 \pm 1.3
Tri-	48.8 \pm 0.2	48.9 \pm 0.1	48.2 \pm 1.1	49.9 \pm 0.6
Tetra-	33.0 \pm 1.3	32.1 \pm 0.2	31.6 \pm 1.1	26.8 \pm 2.1
Penta-	4.9 \pm 0.0	4.3 \pm 0.0	4.7 \pm 0.1	3.5 \pm 0.3
Hexa-	0.7 \pm 0.0	0.6 \pm 0.0	0.6 \pm 0.0	0.5 \pm 0.0

TABLE 5.

Molar percentages of PCBs of different degrees of chlorination after 16 weeks incubation. Values are the means of 2 replicates.

Congeners	14 PPM		140 PPM		700 PPM	
	Live	Auto-claved	Live	Auto-claved	Live	Auto-claved
Mono-	3.4	2.7	16.4	0.0	66.7	4.0
Di-	16.6	15.7	28.0	11.0	21.3	15.3
Tri-	48.8	48.7	38.7	49.3	8.5	49.9
Tetra-	27.4	28.8	14.8	34.6	3.0	26.8
Penta-	3.4	3.6	1.9	4.5	0.5	3.5
Hexa-	0.4	0.4	0.1	0.6	0.0	0.5

**FIGURE 1. TECHNOLOGIES RETAINED FOR
DEVELOPMENT OF REMEDIAL ALTERNATIVES
NEW BEDFORD HARBOR**

NONREMOVAL

- **CAPPING**
- **HYDRAULIC CONTROLS**
 - **EARTHEN EMBANKMENTS**
 - **SHEETPILE**
- **SOLIDIFICATION**

REMOVAL

- **HYDRAULIC DREDGES**
 - **CUTTERHEAD**
- **SPECIAL PURPOSE DREDGES**
 - **REFRESHER**
 - **PNEUMA**
 - **MUDCAT**
- **EXCAVATION**
 - **WATERTIGHT CLAMSHELL**

**TREATMENT
(SEDIMENT)**

- **THERMAL**
 - **INCINERATION**
- **PHYSICAL**
 - **SOLVENT EXTRACTION**
 - **SUPERCRITICAL FLUID EXTRACTION**
 - **SOLIDIFICATION**
 - **VITRIFICATION**
- **CHEMICAL**
 - **ALKALI METAL DECHLORINATION**
- **BIODEGRADATION**

(WATER)

- **DEWATERING**
 - **BELT FILTER PRESS**
 - **GRAVITY THICKENING**
 - **PLATE & FRAME PRESS**
 - **VACUUM FILTRATION**
- **TREATMENT**
 - **COAGULATION/FLOCCULATION/PRECIIPITATION**
 - **SEDIMENTATION**
 - **FILTRATION**
 - **CARBON ADSORPTION**

DISPOSAL

- **IN-HARBOR**
- **SHORELINE**
- **UPLAND**
- **OFF SITE**
- **OCEAN**

FIGURE 2: Example of reductive dechlorination reactions by anaerobic microbial communities.

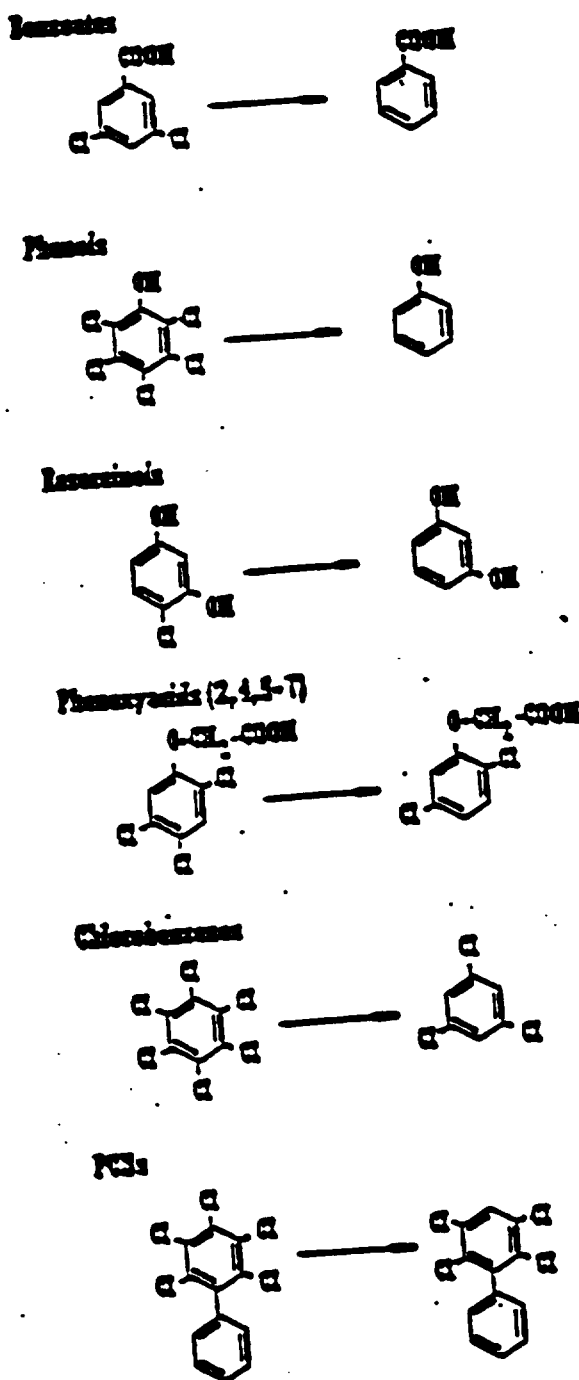


FIGURE 3: Capillary gas chromatograms of PCB samples from the upper Hudson River: (a) Aroclor 1242 standard; (b) Pattern A- from aerobic surface sediments showing depletion of mono- and disubstituted congeners on left of chromatogram; (c) Pattern B- from anaerobic sample showing marked reduction in hexa-, penta-, and some tetrasubstituted congeners (right side of chromatograms) and increase in mono-, di-, and trisubstituted congeners (from Brown et al., 1984).

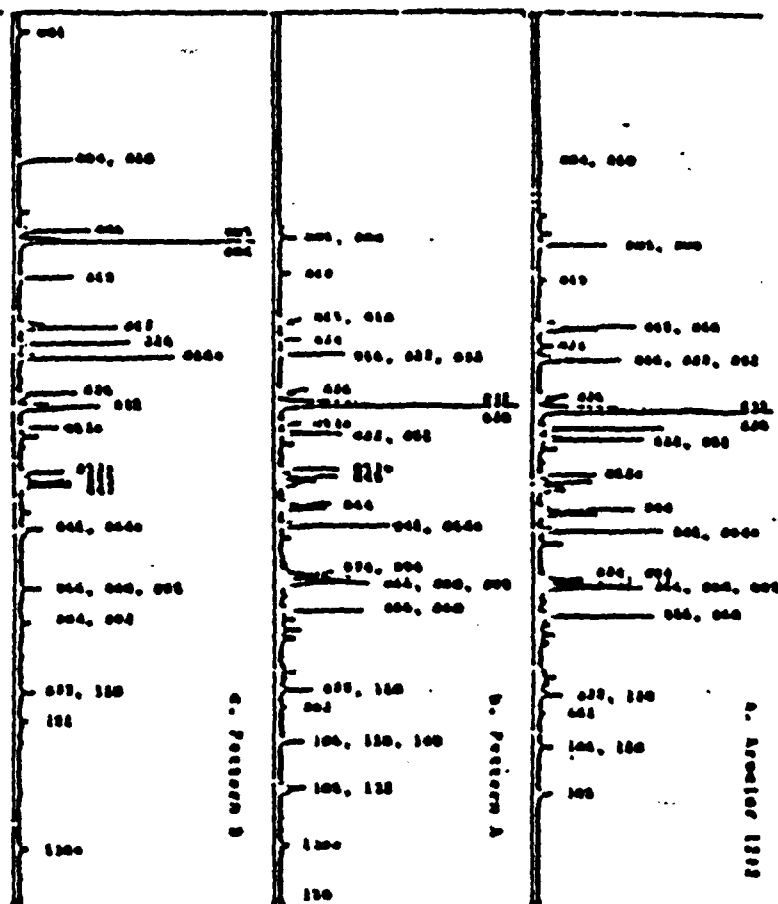


FIGURE 4: Relative increase in the amount of 2,4,2'-CB in the live samples over autoclaved control samples in the Hudson River sediment experiments.

PCB PRODUCT FORMATION (2,4,2')

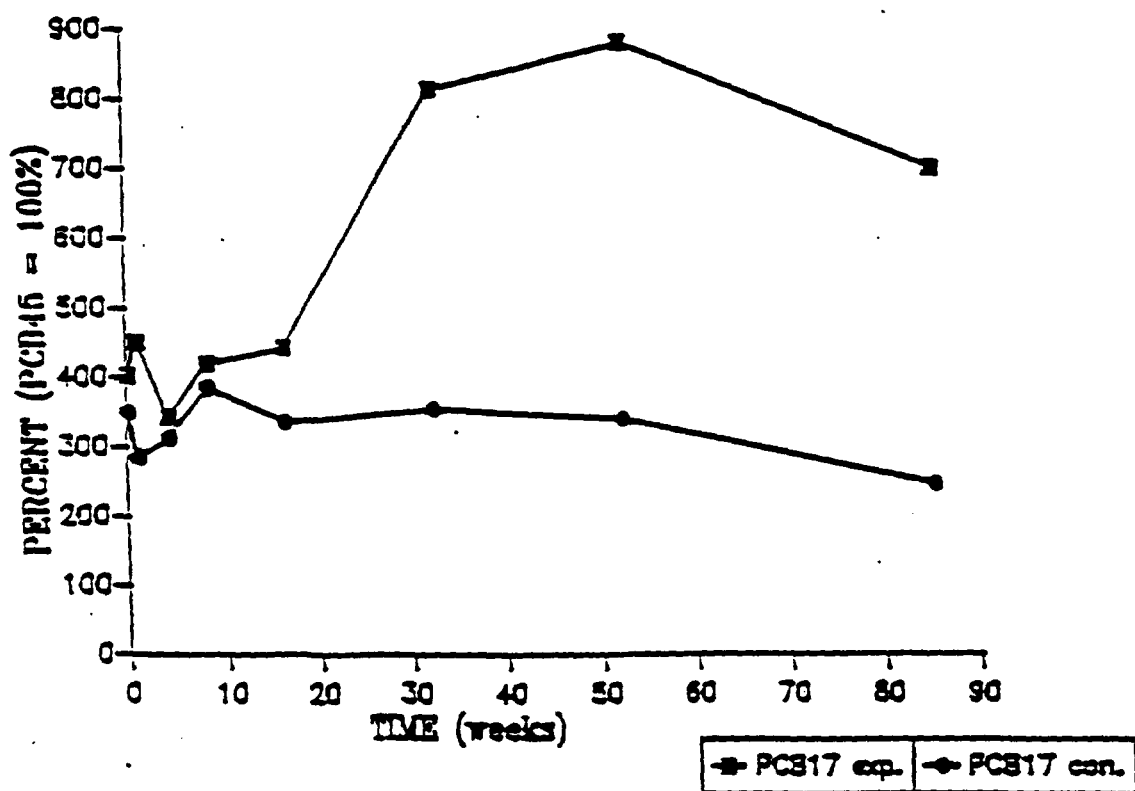
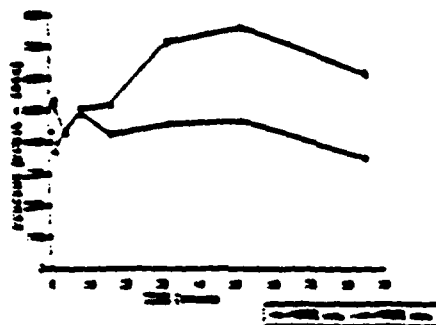
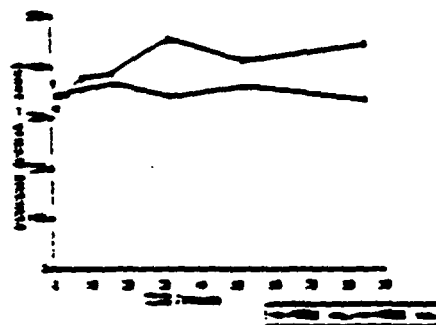


FIGURE 5: Relative increases in the amounts of three trichlorobiphenyls in the live samples over autoclaved control samples in the Hudson River sediment experiments.

PCB 2,3,7-TRICHLOROBIPHENYL (2,3,7)



PCB 2,3,7-TRICHLOROBIPHENYL (2,3,7)



PCB 2,3,7-TRICHLOROBIPHENYL (2,3,7)

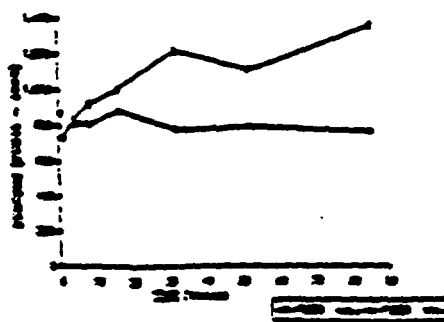


FIGURE 6: Relative changes in the amounts of three tetrachlorobiphenyls between the live samples and autoclaved control samples in the Hudson River sediment experiment.

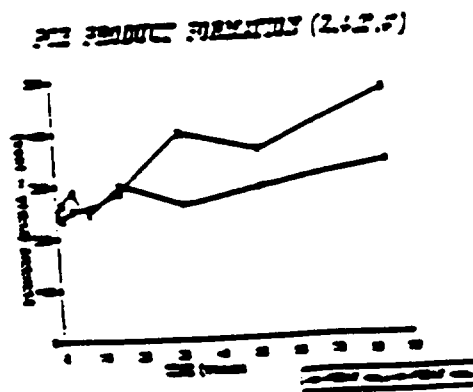
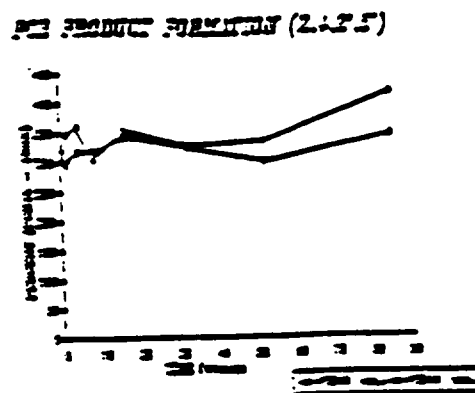
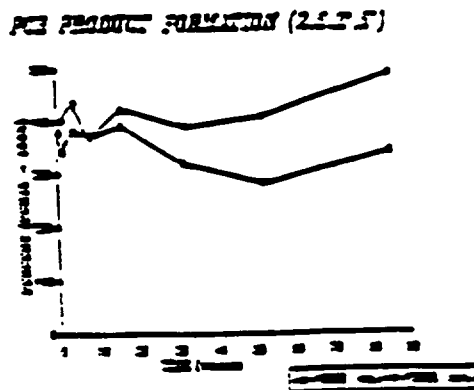
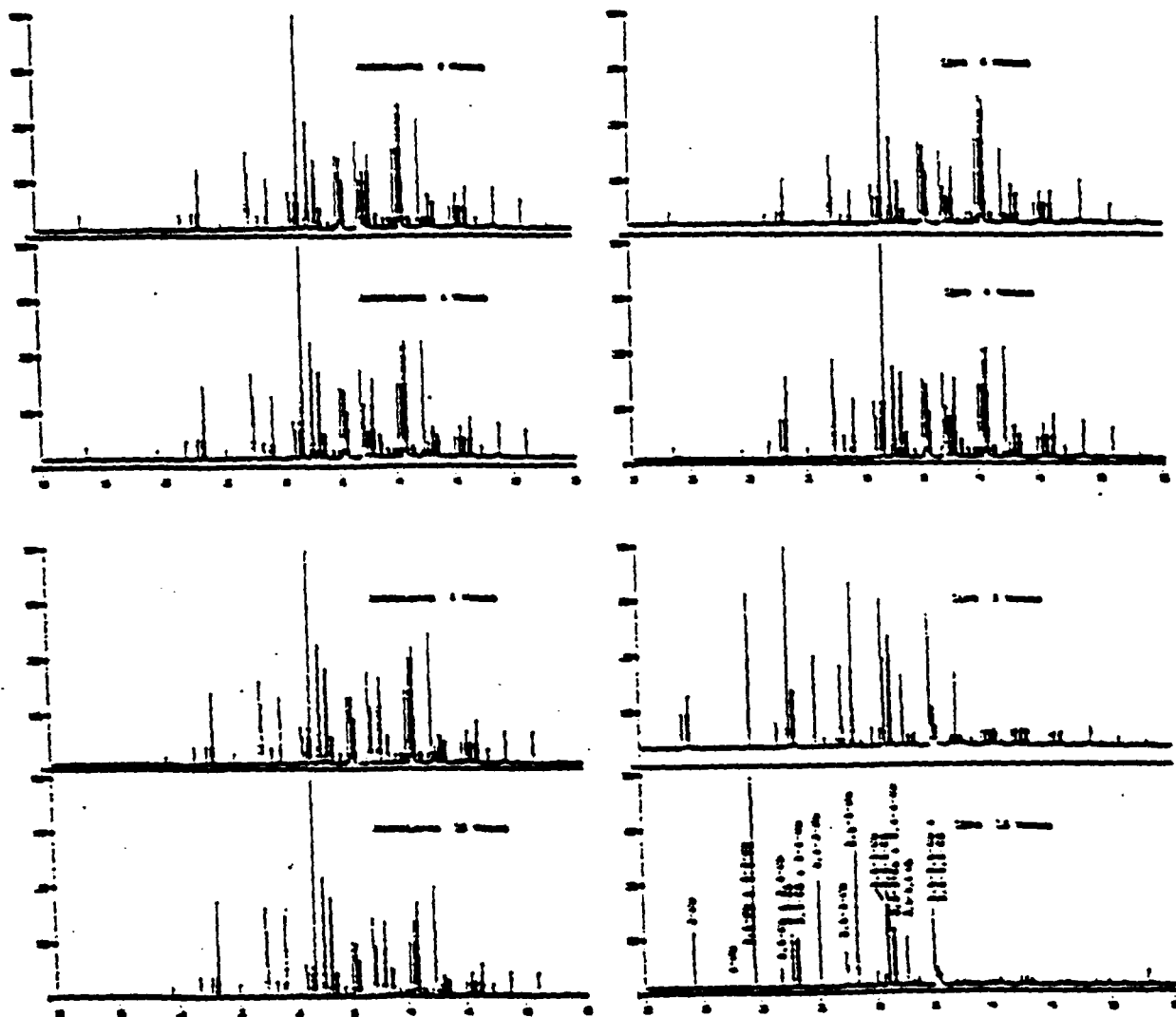


FIGURE 7: Anaerobic reductive dechlorination of Aroclor 1242 in laboratory cultures. These capillary gas chromatograms show extensive dechlorination of the original PCB mixture to yield predominantly ortho-substituted mono- and dichlorobiphenyls.



APPENDIX A

BASIC STUDIES ON CHLOROBENZOATE

DECHLORINATION BY DCB-1

While much practical information can be learned from data on reductive dechlorination in natural samples and mixed communities, the complexity of these samples makes it difficult to understand the basic biological and mechanistic information on the reductive dechlorinating organisms and process. This basic information is important to provide a sound understanding of the process and its requirements, and to underpin the development of reliable, biological dechlorinating systems. The discussion below shows that, contrary to the assertions/implications in the DART, considerable information on biodegradation mechanisms exists.

One bacterium that has been isolated in pure culture that is capable of dechlorination of chloroaromatic substrates is known as strain DCB-1. It is of interest in the context of these comments because it may serve as a model to gain insight into the mechanism of aromatic dehalogenation. DCB-1 converts 3-chlorobenzoate to benzoate and Cl^- . DCB-1 is a slow growing, gramnegative, nonspore forming, obligately anaerobic rod which has an unusual morphological feature--a collar--surrounding every cell. In culture it has an unusually restricted substrate range, growing only on pyruvate, however it seems to be an effective scavenger of a variety of carbon compounds and perhaps exists in nature with this mode of substrate acquisition. DCB-1 also consumes H_2 , which inhibits dechlorination at high concentrations. DCB-1 also fixes CO_2 and probably derives much of its cell carbon by this means. DCB-1 seems to be closely related to

the sulfate reducing class of bacteria in that its growth is stimulated by oxidized sulfur compounds as electron acceptors. It contains desulfoviridin and it has a mixotrophic metabolism. Because it produces sulfide from several oxysulfur anions it is a sulfidogen, however it is important to note that it cannot be maintained on sulfate as an electron acceptor.

From the physiological information DCB-1 appears to obtain its reductant for dechlorination from either H_2 or certain organic compounds, e.g., acetate and pyruvate, and its cell carbon primarily from CO_2 since it cannot metabolize the dechlorination product, benzoate. This information does not explain where DCB-1 obtains its energy. Calculation of Gibbs free energy under the conditions of DCB-1 growth ($\Delta G'$) shows that dechlorination releases considerable energy (Dolfing and Tiedje, 1987):



This energy appears to be productively used by DCB-1 since (i) the dechlorination step resulted in a 38% increase in cell yield (measured as increased protein) over benzoate as a substrate for a defined chlorobenzoate degrading consortium containing DCB-1, and (ii) ATP concentration in cells more than doubled when given chlorobenzoate over that present when the same consortium was given benzoate. This finding is particularly important since it shows why microorganisms may be selected to dechlorinate aromatic compounds--they may gain energy for growth

as well as new electron acceptors; these are usually the most limiting factors for organisms in anaerobic environments.

Thermodynamic calculations of Tiedje and others for most classes of aromatic compounds including the PCBs show that in every case considerable energy is available from each dechlorination event (Brown et al., 1987b, see also, J. Dolfig, K. Harrison, J. Tiedje, unpublished data). Natural selection should work in the direction of selecting organisms or mutant strains that can use this energy for growth and thus it would not be surprising if long-term PCB exposure effected selection of PCB dechlorinating organisms.

APPENDIX B

DEHALOGENATION OF BENZENES

The biological dechlorination of HCBz in anaerobic sludge and sediment has been confirmed in laboratory experiments. Two pathways for HCBz dechlorination in anaerobic sludge were reported by Fathepure et al. (1988). In the major pathway, HCBz was sequentially dechlorinated to pentachlorobenzene, 1,2,3,5-tetrachlorobenzene, and 1,3,5-trichlorobenzene which was not further dechlorinated. These are the same substitution patterns reported for HBBz debromination products in Japanese river sediments (Watanabe et al., 1986). In a minor pathway, HCBz was dechlorinated to all dichlorobenzenes via pentachlorobenzene, 1,2,4,5-tetrachlorobenzene, and 1,2,4-trichlorobenzenes. Similarly, Rhine River sediments were found to dechlorinate HCBz to 1,3,5-trichlorobenzene (Holliger et al., 1988). In this case dechlorination was dependent on the addition of an electron donating substrate (lactate, glucose, ethanol, or isopropanol). Anaerobic upflow columns containing Rhine River sediment were found to dechlorinate all tri- and dichlorobenzenes to monochlorobenzene (Bosma et al., 1988). This required an acclimation period (160 days for 1,3,5-trichlorobenzene), but thereafter all trichlorobenzene (30-50 nmol/l) was removed in the first 2.5 to 5.0 cm through the column. The flow rate was 1 cm/h. The dechlorination of the dichlorobenzenes was inhibited in the presence of 1,3,5-trichlorobenzene or nitrate (35 mg/l) but not sulfate (to 20 nmol/l). This indicates that sulfate does not always inhibit anaerobic dechlorination, but that other electron acceptors (e.g., nitrates) may, depending on the specific

microorganisms involved. Also, as monochlorobenzene is readily degraded aerobically, these experiments suggest that the complete elimination of chlorobenzenes by a combination of anaerobic and aerobic methods is feasible.

APPENDIX C

**ADDITIONAL DETAILS OF THE
TIEDJE AND QUENSEN EXPERIMENTS**

The first of the Tiedje and Quensen experiments involved the addition of five pure PCB congeners (1 ppm each) to Hudson River sediments, as well as to sewage sludge, and chlorophenol-enriched sewage sludge. This experiment also allowed monitoring of the change in existing PCB congeners in the contaminated sediments. The second set of Tiedje and Quensen experiments involved transferring microorganisms from contaminated Hudson River sediments and other river sediments to nonPCB-contaminated sediments and the addition of five pure PCB congeners at concentrations of 1 ppm each. And, the third Tiedje and Quensen experiment involved the same transfer procedure, but Aroclor 1242 was added in concentrations of 14, 140, and 700 ppm on a sediment dry weight basis.

The basic procedure for the first set of experiments consisted of adding a mixture of five PCB congeners (2,3',4,4'-CB, 2,3,4,5,6-CB, 2,2',4,4',6,6'-CB, 2,2',3,4,4',5',6-CB, and 2,2',3,3',4,4',5,5'-CB) in 50 ml of acetone to 50 ml of anaerobic Hudson River sediment or sewage sludge in tightly stoppered serum bottles. Autoclaved treatments served as controls. Samples were taken periodically, extracted with 15% methylene chloride in hexane and analyzed by gas chromatography for the appearance (or increase in size) of peaks associated with the potential primary (single) dechlorination products. Because no primary dechlorination products were ever observed for the octa-CB added, it could be used as an internal standard to look for relative decreases in the amounts of the other congeners added.

The Hudson River sediment showed greater potential to anaerobically dechlorinate PCBs than did fresh sewage sludge or sludge acclimated to each of the monochlorophenols. Of the five PCB congeners added to the sediments, 2,3',4,4'-CB and 2,3,4,5,6-CB decreased 31% and 44%, respectively, during 51 weeks of incubation (Table 1) equivalent to half-lives of 1.8 years and 1.5 years respectively. A peak corresponding to 2,3,4,6-CB and/or 2,3,5,6-CB (coeluting isomers) from the dechlorination of the pentachlorobiphenyl was first detected after eight weeks of incubation. At the end of 51 weeks the tetrachlorobiphenyl formed accounted for 28% of the 2,3,4,5,6-CB originally present. The tetrachlorobiphenyl that was formed was likely further dechlorinated to 2,4,6-CB and 2,6-CB. No primary dechlorination products from the 2,3',4,4'-CB added to the sediments could be detected because of high-background levels (of PCBs) in those sediments and/or poor resolution of the expected trichlorobiphenyls. A small peak corresponding to 2,2',4,4',5,6'-CB, from the dechlorination of 2,2',3,4,4',5'-CB, and representing about 4% dechlorination, was first noted after 32 weeks. There was no evidence for the dechlorination of 2,2',3,3',4,4',5,5'-CB or 2,2',4,4',6,6'-CB by the Hudson River sediments.

The Hudson River sediments used in the above experiment were contaminated with PCBs, making detection difficult for the dechlorinated products from the PCB congeners that were added. (This was the impetus for developing the transfer technique, in which microorganisms are eluted from the contaminated sediments

and transferred to cleaner sediments, used in the experiments that are described below.) But it also unexpectedly allowed the observation of relative changes in the peak sizes for some of the PCBs already in this sediment.

To gain a clearer understanding of the relative changes in the relative peak sizes of the existing PCBs, each of the selected peak areas was plotted against the area of the 2,2',3,6-CB peak over time for the experimental and autoclaved control treatments. The 2,2',3,6-CB peak was chosen because it did not appear to change over time in either the experimental or control treatments.

These plots revealed that the area of the 2,2',4-CB peak doubled between 16 and 32 weeks in the experimental treatments and remained relatively constant thereafter (Figure 4). There was no appreciable increase prior to 16 weeks. There was no change in the 2,2',4-CB peak area over time for the autoclaved controls.

The plots for the trio of peaks corresponding to 2,4',6-CB, 2,3',5-CB, and 2,4',5-CB revealed that all these peaks increased over time in the experimental treatments, but to different degrees (Figure 5). Most of the increase occurred between 16 and 32 weeks. The relative amounts of 2,4',6-CB and 2,4',5-CB increased approximately 50%. The increase of 2,3',5-CB was much less, amounting to approximately 10%. There was essentially no change in relative area for any of these peaks over time for the autoclaved controls.

The plots also revealed an approximately 30% increase in the peak area for 2,2',4,4'-CB in the experimental treatment, again occurring primarily between 16 and 32 weeks (Figure 6). The relative area for the 2,2',5,5'-CB peak decreased nearly 25% between 16 and 51 weeks in the experimental treatments while the 2,2',4,5'-CB peak showed no change. There was little or no change in the relative areas of any of these peaks in the controls. These results show that some of the existing PCBs in the upper Hudson River sediments were dechlorinated during the one-year incubation period under controlled laboratory conditions.

Tiedje and Quensen have also observed dechlorination of 2,3,4,5,6-CB in the second series of experiments employing microorganisms eluted from sediments from the Hudson River (New York), Silver Lake (Massachusetts), and the Pine River (Michigan, contaminated with polybrominated biphenyls, hexabromobenzene, and DDT) and Red Cedar River in Michigan. The Pine River cultures showed the earliest evidence of dechlorination of the 2,3,4,5,6-pentachlorobiphenyl. Approximately 3% of this PCB congener was dechlorinated by the end of four weeks by the Pine River microorganisms (Table 2). The cultures from the three PCB-contaminated sites exhibited longer lag times before dechlorination was observed, but showed the greatest amount of dechlorination by the end of the experiment. The Red Cedar River sediments, with no known history of PCB exposure, showed only 1.3% dechlorination of 2,3,4,5,6-CB at the end of 32 weeks. This is further evidence

that PCB dechlorinating microorganisms can be found at separate sites, but previous exposure to halogenated aromatics may be necessary to select for this activity.

Tiedje and Quensen also recently assessed the ability of microorganisms from PCB-contaminated Hudson River sediments to dechlorinate Aroclor 1242 under anaerobic conditions using the "transfer" experiment technique described above. Each serum bottle contained 50 grams of sieved air dried "clean" sediment, 30 ml of reduced anaerobic mineral medium (RAMM) (Shelton and Tiedje, 1984a), and 50 ml of (RAMM) supernatant containing microorganisms from PCB-contaminated Hudson River sediments. Six of the 12 bottles were autoclaved to serve as controls. Aroclor 1242 in 100 μ l of acetone was added at three different levels (0.7, 7, and 35 mg per bottle), two live treatments and two autoclaved treatments at each level. These Aroclor additions correspond to 14, 140, and 700 ppm on a sediment dry weight basis. Teflon-lined stoppers were used to seal the bottles after the Aroclor additions. The bottles were shaken for 30 minutes after the PCB addition and for 10 minutes prior to each sampling event at 0, 4, 8, and 16 weeks. Samples (approximately 2 ml of slurry) were removed with sterile pipets while flushing with filter sterilized oxygen-free nitrogen/carbon dioxide (80:20, v:v), and bottles were resealed after sampling. The samples were frozen until the time of extraction.

The samples were extracted once with 10 ml of acetone (to remove water) and twice with hexane:acetone (9:1 in volume terms)

by shaking. The acetone was extracted with 2% NaCl in distilled water, and the hexane extract cleaned up using sulfuric acid, mercury, and Florisil. The samples were then analyzed on a capillary gas chromatograph in the laboratory.

The PCB congeners in each of the 60 peaks quantitated are given in Table 3. The percentage of the total PCBs (on a molar basis) represented by each peak was calculated to facilitate comparisons between samples.

Dechlorination of the Aroclor was evident from a simple visual comparison of the chromatograms, especially at the highest PCB concentration (Figure 7). Early eluting peaks, representing the lesser chlorinated congeners, increase with time in the live samples but not in the autoclaved controls. There is a corresponding decrease in the later eluting, more highly chlorinated congeners. Tiedje and Quensen's results for the 8 and 16 week chromatograms for the live samples at 700 ppm Aroclor 1242 are very similar to those for pattern C Hudson River core samples as reported by Brown et al. (1987a, b). Most notable are the accumulation of chlorobiphenyls substituted only at the ortho (2) position. 2-Chlorobiphenyl (2-CB) increases from <1% to 63% in the live samples in the 700 ppm Aroclor treatment, and 2,2'-CB and/or 2,6-CB (coeluting isomers) increase from <0.5% to 14% of the total PCBs. Peak 7 (2,2',6-CB) also increases from 0.4% to 2%.

There are also notable transient increases in peaks 10 (2,2'5-CB and 4,4'-CB) and 18 (2,4',5-CB) at four weeks and for

peak 5 (2,3'-CB) at eight weeks. These congeners are likely first produced by the dechlorination of more highly chlorinated congeners and then subsequently dechlorinated.

The progressive nature of the dechlorination process is evident upon examination of the proportion of mono-, di-, tri-, tetra-, penta-, and hexa-CBs at each sampling time. Molar percentages of PCBs represented by peaks in each of these groupings is presented in Table 4 for averaged data for the treatments at the highest PCB concentration.

Aroclor 1242 contains predominantly tri- and tetra-CBs. In the live samples (Table 4) these are progressively dechlorinated to mono- and di-CBs while there is little change over time in the autoclaved controls.

The effect of PCB concentration on dechlorination is summarized in Table 5. Substantial dechlorination was noted at the highest (700 ppm) concentration while at the lowest concentration (14 ppm) there was little dechlorination at 16 weeks. Of the two replicates receiving 140 ppm of Aroclor 1242, one showed nearly as much dechlorination at 16 weeks as did the 700 ppm treatment. The lesser extent of dechlorination for the 140 ppm treatment as given in Table 5 (average of the two replicates) may, therefore, be a result of longer lag time for one replicate or slower rates at this concentration. Nonetheless, these data show that PCB dechlorination appears to be concentration dependent, occurring much faster at higher concentrations.